HUMAN PLATELET-DERIVED GROWTH FACTOR (PDGF): AMINO-TERMINAL AMINO ACID SEQUENCE

Elucidation of the primary structure of platelet-derived growth factor (PDGF) will help provide an understanding of the molecular basis for its function and its relation to other polypeptide growth factors, serum proteins, and platelet factors. This paper describes the results of amino-terminal amino acid sequence analysis of both active PDGF and the major, inactive peptides derived from it by disulfide reduction. To do this, PDGF obtained from outdated human platelets was subjected to amino-terminal sequence analysis by automated Edman degradation. Despite the apparent presence of limited proteolytic degradation of the protein derived from this method, the sequence analysis revealed two primary peptide sequences and suggests that active PDGF is composed of two, possibly homologous peptides linked by a disulfide bond or bonds.

Antoniades, H. N. and Hunkapiller, M. W.

Science 220:963-965, 1983.

Other support: Weingart Foundation and the National Cancer Institute.

From the Center for Blood Research and Department of Nutrition, Harvard University School of Public Health, Boston, and the Division of Biology, California Institute of Technology, Pasadena.

SIMIAN SARCOMA VIRUS *onc* GENE, v-*sis*, IS DERIVED FROM THE GENE (OR GENES) ENCODING A PLATELET-DERIVED GROWTH FACTOR

Recent investigations of the genetic alterations that cause normal cells to become malignant have focused on a small set of cellular genes. Acute transforming retroviruses have substituted viral genes necessary for replication with these discrete segments of host genetic information. When incorporated within the retroviral genome, these transduced cellular sequences, termed *onc* genes, acquire the ability to induce neoplastic transformation. The profound cellular alterations induced by the activated cellular transforming genes have some similarities to the growth promoting actions of hormones and growth factors. In this paper, it is demonstrated that the transforming protein of a primate sarcoma virus and a platelet-derived growth factor are derived from the same or closely related cellular genes. This conclusion is based on the demonstration of extensive sequence similarity between the transforming protein derived from the simian sarcoma virus *onc* gene, v-*sis*, and a human platelet-derived growth factor. The mechanism by which v-*sis* transforms cells could involve the constitutive expression of a protein with functions similar or identical to those of a factor active transiently during normal cell growth.

Doolittle, R. F., Hunkapiller, M. W., Hood, L. E., Devard, S. G., Robbins, K. C., Aaronson, S. A., and *Antoniades, H. N.*

Science 221:275-277, 1983.

Other support: National Institutes of Health and the Weingart Foundation.

From the Department of Chemistry, University of California, San Diego, La Jolla; Division of Biology, California Institute of Technology, Pasadena; Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, MD; and the Center for Blood Research and Department of Nutrition, Harvard University School of Public Health, Boston.

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Other support: National I
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Bing, R. J.

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HUMAN PLATELET-DERIVED GROWTH FACTOR: STRUCTURE AND FUNCTION

This paper briefly reviews the physicochemical properties of human platelet-derived growth factor (PDGF) and its diverse physiological functions, which include stimulation of cell growth, cell migration, cell metabolism, and modulation of cellular membrane receptors. In summary, PDGF is a heat-stable, cationic polypeptide transported in blood in the α granules of platelets. It is released from platelets during blood clotting. PDGF has been resolved into at least two closely related active polypeptides, PDGF-I and PDGF-II, each consisting of two inactive chains linked together by disulfide bonds. PDGF stimulates the growth of normal cells in culture, including fibroblasts, arterial smooth muscle cells, and glial cells. In addition, PDGF has been shown to stimulate cell migration and many diverse metabolic functions such as amino acid transport, protein synthesis, cholesterol ester synthesis, phospholipid turnover, and prostacyclin synthesis. It modulates receptor binding of other active components such as epidermal growth factor, luteinizing hormone, low-density lipoprotein, and somotomedin C. Specific cell membrane receptors for PDGF have been demonstrated in arterial smooth muscle cells and fibroblasts.

Antoniades, H. N. and Williams, L. T.

Federation Proceedings 42:2630-2634, 1983.

Other support: National Institutes of Health and the Milton Fund, Harvard Medical School.

From the Center for Blood Research and Department of Nutrition, Harvard University School of Public Health, Boston, and the Cardiac Unit, Massachusetts General Hospital, Boston.

THE BIOCHEMICAL BASIS OF MYOCARDIAL FAILURE

In this careful review paper, it can be seen that the findings of more than a century of investigation point to a picture not of a discrete lesion, but rather of the interaction of multiple factors that converge on the common pathway of heart failure. It would seem that the pathways leading to myocardial failure can have a variety of metabolic origins. They can start from disturbances within the myocardium — the processes of energy production in mitochondria, energy utilization by the contractile proteins, or altered calcium flux in one or more of the cellular organelles involved — or they may arise because of faulty β-adrenergic regulation affecting the adrenergic component of contraction. New technology is making it possible to synthesize the contributing factors into a clinical model that will greatly enhance investigators' understanding of these mechanisms. Specifically, the chemical reactions in metabolism need to be followed inside living cells, and indeed, this is now promised through the phenomenon of nuclear magnetic resonance (NMR). As NMR spectroscopy brings the study of cardiac metabolism and biochemistry to the bedside, understanding of fundamental mechanisms of various cardiac disorders, especially of the heart failure state, should greatly increase.

Bing, R. J.

Hospital Practice September 1983, pp. 93, throughout.

From the Huntington Medical Research Institutes, Pasadena, CA.

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Source: https://www.industrydocuments.ucsf.edu/docs/ypxk0000

THE EFFECTS OF A NEW CARDIOTONIC DRUG (TA-064) ON NORMAL AND FAILING HEART PREPARATIONS

Since Withering discovered the action of digitalis in 1785, few drugs have been introduced with prolonged inotropic effect on the failing heart. This report, however, deals with the effect of TA-064, a new positive inotropic compound, on the metabolic and dynamic function of normal and failing perfused working rabbit heart preparations. In order to increase the oxygen capacity of the perfusate, a perfluorocarbon (FC-43) oxygenated in an infant bubble oxygenator was employed. Metabolic measurements included high energy phosphate and glycolytic intermediates. Left ventricular systolic, end-diastolic pressures, dp/dt_{max}, cardiac output, coronary flow, and myocardial oxygen consumption were determined. Administration of sodium pentobarbital (50 to 100 mg) or increased pre- and afterload resulted in depression of myocardial contractility. In the nonfailing heart preparation, single injections of TA-064 resulted in marked positive inotropic effects, increased heart rate and myocardial oxygen consumption. Propranolol in concentrations of 4 ng/ml abolished the positive inotropic effect. These and other observations presented here show that TA-064 is a powerful positive inotropic agent which in the isolated perfused heart increases both heart rate and myocardial oxygen consumption. It does not influence high energy phosphate or glycolytic intermediates in heart muscle.

Bing, R. J. et al.

Current Therapeutic Research 36(1):133-147, 1984.

Other support: The Hoover Foundation and the Lindbergh Fund.

From the Department of Experimental Cardiology, Huntington Medical Research Institutes and Huntington Memorial Hospital, Pasadena, CA.

MICROBUBBLE DYNAMICS VISUALIZED IN THE INTACT CAPILLARY CIRCULATION

Existing techniques for two-dimensional contrast echocardiographic imaging of myocardial tissue rely on the introduction of contrast agent solutions containing microbubbles of air, which alter the acoustic impedance and produce differential echo enhancement. The two methods used in the investigators' laboratory to generate microbubbles in solutions serving as contrast agents included the widely used hand-agitation method and the newer ultrasonic microcavitation (sonication) method. The latter has been demonstrated to generate smaller and more uniform microbubbles in an *in vitro* system. This study was designed to observe, by direct microscopic examination of a cat mesentery preparation, the behavior and fate of the microbubbles in an *in vivo* system. The *in vivo* mesentery observations confirm the critical role of microbubble size in its unhindered passage through the capillary vasculature. The smaller and more uniform sonicated microbubbles passed rapidly through the microcirculation along with the red blood cells, whereas the larger microbubbles were observed to coalesce and interrupt the flow of blood and subsequently collapse or shrink.

Feinstein, S. B., Shah, P. M., *Bing, R. J.*, Meerbaum, S., Corday, E., Chang, B-L., Santillan, G., and Fujibayashi, Y.

Journal of the American College of Cardiology 4(3):595-600, 1984.

Other support: Americ
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Research Foundation.

From the Huntington M
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-600, 1984.

Other support: American Heart Association, The Hoover Foundation, the Medallion Group of Cedars-Sinai Medical Center, the Keck Foundation, and the Eliot Corday Research Foundation.

From the Huntington Medical Research Institutes, Pasadena, CA, and the Division of Cardiology, Department of Medicine, Cedars-Sinai Medical Center, the University of California at Los Angeles School of Medicine and the Wadsworth Veterans Administration Medical Center, Los Angeles.

EFFECT OF SEROTONIN ON ALBUMIN AND LOW DENSITY LIPOPROTEIN UPTAKE IN PERFUSED RABBIT FEMORAL ARTERIES

Transport of blood plasma constituents into the arterial wall has considerable clinical implication. Increased intraluminal pressure, turbulence and shear stress are important factors in the production of vascular injury to the endothelium. Thus, uptake of low density lipoprotein (LDL) plays an important role in the development of atherosclerosis. Serotonin and other vasoactive material may result in changes in the arterial wall leading to increased uptake of albumin or LDL. The present study was conducted to determine the effect of serotonin (10^{-5} M 5-hydroxytryptamine) and serotonin-induced arterial constriction on albumin and LDL uptake rates in perfused rabbit femoral arteries. The results show that the presence of serotonin inhibits the rate of uptake of both albumin and LDL. The effect on albumin uptake does not have a direct dose response dependence and is linearly dependent on transmural pressure. In contrast, LDL uptake rates are only slightly affected by pressure. Thus, albumin and LDL uptake processes appear to be due to separate mechanisms.

Santillan, G. G., Sato, M. and *Bing, R. J.*

Angiology 35(11):694-700, 1984.

Other support: The Hoover Foundation and The Lluella Morey Murphy Foundation.

From the Huntington Medical Research Institutes and Huntington Memorial Hospital, Pasadena, CA.

RED CELL VELOCITY AND AUTOREGULATION IN THE CEREBRAL CORTEX OF THE CAT

This report compares the effect of changes in blood pressure on blood flow with those in red cell velocity in the cerebral cortical microcirculation of the cat. To do this, total and regional blood flows were measured during a steady-state with radioactive microspheres; a newly devised method, based on transillumination, was employed for direct visualization of the microcirculation in the cerebral cortex. Craniotomy failed to influence either autoregulation or red cell velocity under the skull opening or of the total cerebral flow. Autoregulation of total and regional cerebral flow was present. In contrast, marked pressure-dependent variations in microcirculatory red cell velocity occurred. A fall in systemic pressure was accompanied by a decrease in red cell velocity; return of pressure resulted in increased red cell velocity and in some instances, in an overshoot. Considerable inhomogeneity of red cell velocity in different microcirculatory vessels was encountered. In some cases a fall in systemic pressure led to complete disappearance of red cells.

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Source: https://www.industrydocuments.ucsf.edu/docs/ypxk0000

Chang, B. L., Santillan, G. and *Bing, R. J.*

Brain Research 308:15-24, 1984.

Other support: The Hoover Foundation and The Lluella Morey Murphy Foundation.

From the Huntington Medical Research Institutes and the Huntington Memorial Hospital, Pasadena, CA.

ANTIBODIES TO THE HEPARAN SULFATE PROTEOGLYCANS SYNTHESIZED BY ENDOTHELIAL CELL CULTURES

In this paper, some of the properties of an antiserum raised against the heparan sulfate proteoglycans are described, and the implications of these findings with respect to an understanding of the biological significance of endothelial cell proteoglycans are discussed. The cell lines used for these studies, an endothelial cell line and a subendothelial cell line, were derived from the intima of rabbit aorta. Using polyacrylamide gel electrophoresis, it was seen that endothelial cell cultures derived from rabbit aorta synthesize a wide spectrum of heparan sulfate proteoglycans. The electrophoretically slower-moving heparan sulfate proteoglycans have been isolated from the endothelial cell culture medium. Antibodies to these proteoglycans species have been raised in the goat. The goat antiserum binds selectively the heparan sulfate proteoglycans that were used for the immunization and does not cross-react with the other (faster moving) species. Only a moderate level of cross-reactivity was observed with the heparan sulfate proteoglycans synthesized by another cell line (presumably smooth muscle cells) of vascular derivation. These results support the suggestion that structural differences in the heparan sulfate proteoglycans are responsible for certain differences in function between the various cell types of the vessel wall.

Buonassisi, V. and Colman, P.

Biochimica et Biophysica Acta 760:1-12, 1983.

Other support: National Institutes of Health.

From the Department of Biology Q-058, University of California, San Diego, La Jolla.

HUMAN PLASMA KALLIKREIN RELEASES NEUTROPHIL ELASTASE DURING BLOOD COAGULATION

Granules from viable human neutrophils contain a variety of enzymes known to be released into the extracellular environment. One of the more important granules is elastase, which may be responsible for proteolysis of vital structures in the lung. Since human plasma kallikrein has been shown to stimulate neutrophil chemotaxis, aggregation and oxygen consumption, the ability of kallikrein to release neutrophil elastase was investigated. Neutrophils were isolated by dextran sedimentation, and elastase release was measured by both an enzyme-linked immunosorbent assay and an enzymatic assay using *t*-butoxy-carbonyl-Ala-Ala-Pro-Val-amino methyl coumarin as the substrate. Kallikrein, 0.1-1.0 U/ml, was incubated with neutrophils that were preincubated with cytochalasin B (5μg/ml). The release of elastase was found to be proportional to the kallikrein concentration. Kallikrein released a maximum of 34% of the total elastase content. In a series of experiments carried out to determine if kallikrein

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Other support: National I
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was a major enzyme involved in neutrophil elastase release during blood coagulation, 2.75μg of elastase were released when 10 million neutrophils were incubated in 1ml of normal plasma in the presence of 30 mM $CaCl_2$ for 90 min. In contrast, neutrophils incubated in prekallikrein-deficient or Factor XII-deficient plasma released less than half that amount of elastase. The addition of purified prekallikrein to prekallikrein-deficient plasma restored neutrophil elastase release to normal levels. Moreover, release of elastase was enhanced in plasma deficient in C1-inhibitor, the major plasma inhibitor of kallikrein. These and other data suggest that kallikrein may be a major enzyme responsible for the release of elastase during blood coagulation.

Wachtfogel, Y. T., Kucich, U., James, H. L., Scott, C. F., Schapira, M., Zimmerman, M., Cohen, A. B., and *Colman, R. W.*

Journal of Clinical Investigation 72:1672-1677, 1983.

Other support: National Institutes of Health and the Swiss National Science Foundation.

From the Thrombosis Research Center and the Hematology-Oncology and Pulmonary Sections, Department of Medicine, Temple University School of Medicine, Philadelphia; Graduate Hospital Research Center, Division de Rheumatologie, Hôpital Cantonal Universitaire, Geneva, Switzerland; Merck Sharp & Dohme Laboratories, Rahway, NJ.

CLEAVAGE OF HUMAN HIGH MOLECULAR WEIGHT KININOGEN MARKEDLY ENHANCES ITS COAGULANT ACTIVITY: EVIDENCE THAT THIS MOLECULE EXISTS AS A PROCOFACTOR

The present report demonstrates that intact high molecular weight (HMW)-kininogen is a procofactor that is activated by kallikrein. To be precise, HMW-kininogen, the cofactor of contact-activated blood coagulation, accelerates the activation of Factor XII, prekallikrein, and Factor XI on a negatively charged surface. Although prekallikrein and Factor XI circulate as a complex with HMW-kininogen, no physical association has been demonstrated between Factor XII and HMW-kininogen, nor has the order of adsorption to surfaces of these proteins been fully clarified. In this report, the investigators explore the requirements for adsorption of HMW-kininogen to a clot-promoting surface (kaolin) in purified systems as well as in normal plasma and plasma genetically deficient in each of the proteins of the contact system. In normal plasma, the adsorption of HMW-kininogen to kaolin increased as the quantity of kaolin was increased in the incubation mixture. However, the HMW-kininogen in Factor XII-deficient plasma did not absorb appreciably to kaolin. Furthermore, the quantity of HMW-kininogen from prekallikrein-deficient plasma that adsorbed to kaolin was decreased as compared with normal plasma. Since kallikrein, which is known to cleave HMW-kininogen, is generated when kaolin is added to plasma, the hypothesis was tested that proteolysis by kallikrein was responsible for the enhanced adsorption of HMW-kininogen to kaolin. The observations reported here indicate that HMW-kininogen is a procofactor that is activated by kallikrein, a product of a reaction which it accelerates. This cleavage, which enhances its association with a clot-promoting surface in a plasma environment, is an event that is necessary for expression of its cofactor activity.

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Source: https://www.industrydocuments.ucsf.edu/docs/ypxk0000

Scott, C. F., Silver, L. D., Schalira, M., and *Colman, R. W.*

Journal of Clinical Investigation 73:954-962, 1984.

Other support: National Institutes of Health and the Swiss National Science Foundation.

From the Thrombosis Research Center and the Hematology-Oncology Section of the Department of Medicine, Temple University Health Sciences Center, Philadelphia.

PERSPECTIVES: SURFACE-MEDIATED DEFENSE REACTIONS: THE PLASMA CONTACT ACTIVATION SYSTEM

Observations recorded in this review paper show that, during the past two decades, considerable evidence has accrued that many of the body defenses against injury, specifically blood coagulation, the inflammatory response, classical complement pathway activation and fibrinolysis, are initiated by a common mechanism. Four proteins, namely Factor XII, prekallikrein, high molecular weight kininogen, and Factor XI, have been shown to be the major proteins required to initiate, amplify, and propagate surface-mediated defense reactions by activating $C\bar{1}$, Factor VII, prorenin and plasminogen, by stimulating neutrophils and by releasing bradykinin. Purification of each has allowed functional and immunochemical assays to be developed. The zymogens, XII, PK, and XI_1 are converted by limited proteolysis into the active serine proteases XII_a, kallikrein, and XI_a. High molecular weight kininogen is a nonenzymatic cofactor. The molecular events occurring during *in vitro* contact activation have begun to be described and the role of these pathways in certain diseases has been tentatively probed. Despite advances, new questions have been raised in tandem with the answers provided. Several clinical examples of abnormalities of the contact activation system indicate the need for further application of biochemical knowledge and immunochemical techniques to develop more sensitive assays for activation of the contact system. Emphasis should be shifted from examining the participation of this system in hemostasis to the role of surface-mediated reactions as a defense system in inflammatory diseases.

Colman, R.W.

Journal of Clinical Investigation 73:1249-1253, 1984.

Other support: National Institutes of Health and SCOR on Thrombosis.

From the Thrombosis Research Center and Hematology-Oncology Section of the Department of Medicine, Temple University School of Medicine, Philadelphia.

INACTIVATION OF FACTOR XII ACTIVE FRAGMENT IN NORMAL PLASMA: PREDOMINANT ROLE OF $C\bar{1}$-INHIBITOR

This study indicates that $C\bar{1}$-inhibitor is the major inhibitor of Factor XIIf in normal human plasma. This conclusion is supported by (a) the analysis of the kinetics of Factor XIIf inactivation in purified systems and in prekallikrein-deficient plasma, and (b) the quantitation by sodium dodecyl sulfate polyacrylamide gel electrophoresis

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de Agostini, A., Lijnen,

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(SDS-PAGE) of the Factor XIIf-inhibitor complexes formed in various plasma as the result of the inactivation of purified radiolabeled enzyme. Specifically, in purified systems, the bimolecular rate constants for the reactions of Factor XIIf with $C\bar{1}$-inhibitor, α_2-antiplasmin, and antithrombin III were 18.5, 0.91, and 0.32 $\times 10^4$ M^{-1} min^{-1}, respectively. Furthermore, SDS-PAGE analysis revealed that 1:1 stoichiometric complexes were formed between ^{125}I-Factor XIIf and each of these three inhibitors. In contrast, kinetic and SDS-PAGE studies indicated that Factor XIIf did not react with α_1-antitrypsin or α_2-macroglobulin. In prekallikrein-deficient plasma, the inactivation rate constant of Factor XIIf was 14.4 $\times 10^{-2}$/min^{-1}, a value that was essentially identical to the value predicted from the studies in purified systems. After inactivation in a normal plasma, 74% of the active ^{125}I-Factor XIIf was found to form a complex with $C\bar{1}$-inhibitor, whereas 26% of the enzyme formed complexes with α_2-antiplasmin and antithrombin III. Other observations presented in this study quantitatively demonstrate the dominant role of $C\bar{1}$-inhibitor in the inactivation of Factor XIIf in the plasma milieu.

de Agostini, A., Lijnen, H. R., Pixley, R. A., *Colman, R. W.*, and Schapira, M.

Journal of Clinical Investigation 73:1542-1549, 1984.

Other support: National Institutes of Health and the Swiss National Science Foundation.

From the Division de Rheumatologie, Hôpital Cantonal Universitaire, Geneva, Switzerland; Center for Thrombosis and Vascular Research, Department of Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium; and Hematology-Oncology Section, Department of Medicine, and Thrombosis Research Center, Temple University School of Medicine, Philadelphia.

EFFECTS OF DETERGENTS ON THE ACTIVITY OF α_2-MACROGLOBULIN-PROTEASE COMPLEXES

The enzymatic activity of complexes of proteases, particularly chymotrypsin and elastase, with human α_2-macroglobulin (α_2M) in the presence and absence of detergents such as sodium dodecyl sulfate (SDS) and long-chain fatty acids is reported in this paper. It was found that although α_2M human leukocyte elastase (HLE) complexes have little or no elastolytic activity in the absence of detergents, elastolytic activity becomes significant in their presence at concentrations approximating that of naturally occurring fatty acids in plasma. As results show, when assayed in the presence of detergents such as SDS or sodium lineolate, apparent elastolytic activity of HLE increases almost three-fold, reaching a maximum at a detergent concentration of 1-2 mM. If the HLE is pretreated with an excess of α_2M such that in the absence of detergent all elastolytic activity is lost, and then assayed in the presence of increasing concentrations of either SDS or sodium linoleate, apparent elastolytic activity increases to that of free HLE determined in the absence of detergent. The stimulatory effects of detergents appear to be specific for the α_2M-HLE complex as SDS, sodium lineolate, and sodium oleate inhibited amidase activity of both free and complexed HLE. In each instance, the detergent concentration to reduce amidase activity was approximately 50 μM. These data are consistent with a model in which proteolytic activity is associated with the α_2M-protease complex rather than one in which proteo-

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Source: https://www.industrydocuments.ucsf.edu/docs/ypxk0000

lytic activity is due to dissociated enzyme. The authors suggest this phenomenon may be important in the pathogenesis of emphysema and other inflammatory diseases.

Finlay, T. H. et al.

Annals of the New York Academy of Sciences 421:340-350, 1983.

From the Department of Obstetrics and Gynecology, New York University Medical Center, New York, and the Department of Medicine and Chest Service, Bellevue Hospital, New York.

ACTIVE INTERMEDIATES AND CARCINOGENESIS

In the study presented here, the neoplastic transformation of Balb/c 3T3 cells has been shown to be induced by diethylstilbestrol, estrone, ethylnylestradiol, and estradiol. Moxestrol and estriol, which are poor substrates for catechol estrogen biosynthesis, were not active as transforming agents of these cells. The relative rates of catechol estrogen formation, with ethynylestradiol>estradiol>11β-methylestradiol>11β-ethylestradiol>moxestrol, paralleled the observed frequency of neoplastic transformation. The fact that moxestrol and the 11β-methyl and 11β-ethyl analogs are more potent estrogens *in vivo* than estradiol supports the investigators' hypothesis that the oncogenic potential of an estrogen is related to its relative rate of catechol estrogen formation by the microsomal activating system of the target cells rather than to its activity as a hormone. Also, three of the possible α,β-unsaturated epoxyenone intermediates of estradiol metabolism have been synthesized from 19-nortestosterone and unambiguously characterized. These epoxyenones are approximately 10 to 20 times more potent than estradiol as transforming agents. It is not yet known if their transforming ability is attributable to their direct reaction with DNA to form DNA adducts or to their intracellular conversion to catechol estrogens before DNA damage presumably occurs. In addition, it was seen here that the microsomal activating system of a continuously cultured line of human mammary tumor cells can synthesize catechol estrogens. This MCF-7 cell activating system has been used in these researchers' efforts to isolate an activated intermediate of estradiol metabolism. Their preliminary results provide evidence for the formation of a metabolite which, after acetylation, has the chromatographic mobility of 17β-acetoxy-1β,2β-epoxyestra-4-en-3-one.

Purdy, R. H., Goldzieher, J. W., *Le Quesne, P. W.*, Abdel-Baky, S., Durocher, C. K., Moore, P. H., Jr., and Rhim, J. S.

In: Merriam, G. R. and Lipsett, M. B. (eds.): *Catechol Estrogens*, New York: Raven Press, 1983, pp. 123-140.

Other support: National Cancer Institute, American Cancer Society, and the Joe and Jessie Crump Fund of Fort Worth, TX.

From the Department of Organic and Biological Chemistry, Southwest Foundation for Research and Education, San Antonio, TX; Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston; Department of Chemistry, Northeastern University, Boston; and Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, MD.

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ASSOCIATION OF FIBRIN WITH THE PLATELET CYTOSKELETON

It has been previously postulated that surface membrane proteins become specifically associated with the internal platelet cytoskeleton upon platelet activation. Four lines of evidence support this general hypothesis since the same research group now shows that platelet surface receptors for fibrin become specifically associated with the platelet Triton-insoluble cytoskeleton. (1) Fibrin was detected immunologically in the washed Triton-insoluble cytoskeletons of thrombin-activated platelets under conditions where fibrin polymerization and resultant precipitation was blocked with Gly-Pro-Arg-Pro, a synthetic peptide that inhibits polymerization of fibrin monomer. (2) Radiolabeled fibrin bound to thrombin-activated platelets and became associated with the cytoskeleton. (3) The amount of radiolabeled fibrin bound to thrombin-activated thrombasthenic platelets and their cytoskeletons amounted to about 20% of the fibrin bound to thrombin-activated control platelets and their cytoskeletons. (4) The association of fibrin with cytoskeletons and with the platelet surface was nearly quantitatively blocked by an antibody prepared against cytoskeletons (anti-C), an antibody against isolated membranes of pronase-treated platelets (anti-MI), and a monoclonal antibody to the platelet surface glycoprotein complex, $GPII_b$-GPIII (anti-GPIII). These antibodies blocked ADP and thrombin-induced platelet aggregation as well as thrombin-induced clot retraction. Analysis of the immunoprecipitates obtained with anti-C, anti-MI, and anti-GPIII from detergent extracts of ^{125}I-surface labeled platelets revealed that these antibodies recognized $GPII_b$-GPIII. These data suggest that thrombin activation of platelets results in the specific association of fibrin with the platelet cytoskeleton, that this association may be mediated by the $GPII_b$-GPIII complex, and that these mechanisms may play an important role in platelet aggregation and clot retraction induced by thrombin.

Tuszynski, G. P., Kornecki, E., Cierniewski, C., Knight, L. C., Koshy, A., Srivastava, S., *Niewiarowski, S.*, and Walsh, P. N.

The Journal of Biological Chemistry 259(8):5247-5254, 1984.

Other support: Department of Health and Human Services.

From the Thrombosis Research Center, Temple University School of Medicine, Philadelphia.

INHIBITION OF FIBRINOGEN RECEPTOR-MEDIATED PLATELET AGGREGATION BY HETEROLOGOUS ANTI-HUMAN PLATELET MEMBRANE ANTIBODY: SIGNIFICANCE OF AN M_r = 66,000 PROTEIN DERIVED GLYCOPROTEIN IIIa

It is generally accepted that the major physiological role of fibrinogen is related to its conversion to fibrin that leads to the formation of blood clots and to the arrest of bleeding. In the study reported here, heterologous anti-human platelet membrane antisera were raised in rabbits against membranes prepared from human intact or chymotrypsin- or pronase-treated platelets. Anti-intact anti-chymotrypsin and anti-pronase-treated platelet membrane antibodies (IgG and Fab fragments) inhibited the fibrinogen-induced aggregation of ADP-stimulated and of chymotrypsin-treated platelets. The specific binding of ^{125}I-fibrinogen to these platelets was also inhibited. As revealed by the sodium dodecyl sulfate-polyacrylamide gel electrophoresis of ^{125}I-surface-radiolabeled platelet proteins and by the electrophoresis of the immunoprecipi-

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Source: https://www.industrydocuments.ucsf.edu/docs/ypxk0000

tates prepared from these labeled proteins, predominant components on the surface of intact platelets were glycoproteins IIb and IIIa. These proteins were immunoprecipitated by all three antibodies. In addition, chymotrypsin-treated platelets contained an $M_r = 66,000$ protein on their surface that was also immunoprecipitated by the three types of anti-platelet membrane antibodies. The appearance of this $M_r = 66,000$ protein on the surface of chymotrypsin-treated platelets correlated with the exposure of fibrinogen receptors on the platelet surface as evidenced by the increased platelet aggregation and the enhanced ^{125}I-fibrinogen binding shown by chymotrypsin-treated platelets.

Other related studies suggested that the $M_r = 66,000$ protein was a proteolytic cleavage product of glycoprotein IIIa. Analysis on sodium dodecyl sulfate gels of the major chymotryptic cleavage products of partially purified glycoprotein IIIa and analysis of the peptide of glycoprotein IIIa immunoprecipitated by anti-pronase-treated platelet membrane antibody revealed that the $M_r = 66,000$ protein produced by chymotrypsin on the platelet surface was the major chymotryptic cleavage product of glycoprotein IIIa. A hypothesis was proposed that the $M_r = 66,000$ is a part of glycoprotein IIIa present on the surface of proteolytically treated platelets and it may function in fibrinogen binding and fibrinogen-induced platelet aggregation and that the 66,000-dalton region of glycoprotein IIIa in intact platelets may represent the fibrinogen-binding domain of glycoprotein IIIa.

Kornecki, E., Tuszynski, G. P., and *Niewiarowski, S.*

The Journal of Biological Chemistry 258(15):9349-9356, 1983.

Other support: National Institutes of Health, National Research Service Award and the Kroc Foundation.

From the Thrombosis Research Center, Temple University Health Sciences Center, Philadelphia.

COMPARISON OF PLATELET FIBRINOGEN RECEPTORS ON INTACT AND PROTEOLYTICALLY-TREATED PLATELETS BY USE OF AN ANTI-GLYCOPROTEIN IIIa MONOCLONAL ANTIBODY (MA 123)

The abstract of this paper notes that a murine monoclonal antibody (MA 123) was selected by screening 153 supernatants of hybridoma cells secreting anti-human platelet antibodies for their ability to inhibit the fibrinogen-induced aggregation of chymotrypsin-treated platelets. MA 123 inhibited the binding of ^{125}I-fibrinogen to ADP-stimulated intact human platelets and to platelets treated with chymotrypsin or pronase. Moreover, it inhibited the fibrinogen-induced aggregation of these platelet suspensions. The degree of inhibition was similar in each of the three types of platelets tested. The interactions of MA 123 with the ^{125}I-labeled surface components of intact and chymotrypsin-treated platelets were studied by immunoprecipitation using *Staphylococcus aureus* coated with goat anti-mouse IgG, followed by SDS-polyacrylamide gel electrophoresis and autoradiography. MA 123 precipitated the glycoprotein IIb-glycoprotein IIIa (GPIIb-GPIIIa) complex from the surface of detergent solubilized intact human platelets; it also precipitated GPIIIa from the surface of chymotrypsin-treated platelets. Partially purified GPIIIa was also immunoprecipitated by MA 123. The data suggest that the exposure of fibrinogen receptors by ADP, chymotrypsin or pronase is associated with alterations of GPIIIa on the platelet surface.

Kornecki, E., Lee,
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Source: https://www.industrydocuments.ucsf.edu/docs/ypxk0000

Kornecki, E., Lee, H., Merlin, F., Hershock, D., Tuszynski, G. P., and *Niewiarowski, S.*

Thrombosis Research 34:35-49, 1984.

Other support: National Institutes of Health and a Special Investigatorship Award from the American Heart Association, Southeastern Pennsylvania Chapter.

From the Thrombosis Research Center, Temple University Health Sciences Center, Philadelphia, and Centre National de Transfusion Sanguine, Paris, France.

SURFACE PROPERTIES OF PULMONARY ENDOTHELIAL CELLS

The authors' long-term goal is to help elucidate the means by which pulmonary endothelial cells participate in establishing the quality of systemic arterial blood. Their special interests are in the cellular and molecular means by which the cells actively process polypeptide hormones, prohormones and other excitatory substances so as to determine which pass on to distant target tissues and which do not. It seems true that the researchers' understanding of pulmonary endothelium as a metabolically active and responsive tissue will depend in large measure on understanding the detailed topography of the endothelial surface in terms of relationships between enzymes, binding sites and extracellular material. Reported here is a method for obtaining surface replicas of endothelial cells showing previously unrecognized structures and the distribution of two known surface enzymes. For these experiments, endothelial cells (bovine pulmonary artery or rabbit pulmonary microvascular) that had never been exposed to proteolytic enzymes were seeded onto glass coverslips in flasks or Lux 8-well dishes. Electron microscope immunocytochemistry and routine transmission electron microscopy were undertaken and assays for angiotensin-converting enzyme and carboxypeptidase N were carried out. From the electron micrographs of surface replicas, results show that it is evident that the endothelial cell surface is far more complex than recognized previously. The new surface replicas show structure on what was previously believed to be ultrastructure. Apparently, the glycocalyx itself occurs as a regular carpet on unreacted cells. Overall, the microstructure, fine structure and ultrastructure of the endothelial cell surface appear well-designed to facilitate interactions between plasma solutes with the cell-bound peptidase enzymes. Using the techniques described here virtually the entire surface of an endothelial monolayer in culture can be replicated and the replicas then examined at the resolution of the transmission electron microscope. The authors believe that this surface replication technique has promise for a wide range of studies, not the least of which are those designed to define at the macromolecular level the precise sites of surface enzymes and possibly hormone receptors.

Ryan, U. S. and Ryan, J. W.

In: *Surface Phenomena in Hemorrheology: Their Theoretical, Experimental and Clinical Aspects*, Annals of the New York Academy of Science 416:441-456, 1983.

Other support: National Institutes of Health.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

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Source: https://www.industrydocuments.ucsf.edu/docs/ypxk0000

INFLAMMATORY MEDIATORS, CONTRACTION AND ENDOTHELIAL CELLS

Pulmonary endothelial cells possess on their luminal surfaces a number of enzymes capable of processing circulating vasoactive substances. Among these are enzymes that can metabolize putative inflammatory mediators such as the kinins and the anaphylatoxins. The activities of these enzymes presumably serve to protect the systemic circulation from damage by inflammatory mediators and may also act to protect the endothelial cells themselves. In addition to degradative enzymes, the endothelial surface is equipped with receptors for some inflammatory mediators and other excitatory substances, and receptor activation elicits cellular responses. Efforts to elucidate further the cellular and molecular means by which pulmonary endothelial cells process and respond to circulating hormones, mediators and related substances will require understanding not only of the topography of surface enzymes and receptors but also of the intracellular events occurring as part of the integrated cellular response. In the concluding section of this paper, the authors maintain that what they have tried to convey is that structure and function, indeed dysfunction, of endothelium are highly complex. As subthemes, they hope they have made it evident that kinins very likely are not humoral mediators of acute inflammation and this is in spite of their ability, in pharmacologic doses, to increase microvascular permeability and induce a vasodilation in many ways typical of that seen in inflammation. As another subtheme, they hope they have made clear that inhibitors of CPN can make some inflammatory responses very much more severe. Finally, the investigators believe that interactions of endothelium *in vivo* with Clq and, under certain circumstances, C3b and the Fc segment of IgG may well bespeak means by which endothelial cells become active participants in the acute inflammatory response.

Ryan, U. S. and Ryan, J. W.

In: Courtice, F. C., Garlick, D. G. and Perry, M. A. (eds.) *Progress in Microcircular Research*, Sydney: Committee in Postgraduate Medical Education, University of New South Wales, 1984.

Other support: U. S. Public Health Service.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

ENDOTHELIAL SURFACE ENZYMES AND THE DYNAMIC PROCESSING OF PLASMA SUBSTRATES

This article focuses primarily on reactions between endothelial surface peptidase enzymes and circulating oligopeptides. These reactions are fairly well understood and appear to provide new insights into subjects as diverse as blood pressure control and mechanisms of induction of noncardiogenic pulmonary edema. Furthermore, some of the technology developed to clarify understanding of the immediate metabolic fate of angiotensin I and bradykinin appears to have potential for monitoring both the progress of disease states such as adult respiratory distress syndrome and some forms of drug therapy. One section of this paper is devoted to Fine Structure of the Surface Endothelium: (A) Endothelial Projection; (B) Globular Particles Seen by Freeze-Fracture;

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Dotan, I. and *Shechter, I*

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(C) Surface Antigens; and (D) Surface Replicas. A second section covers Assay of Surface Enzyme *in Vivo*: (A) Substrates; (B) Target Enzymes; (C) Assay Procedure; (D) Biochemical Aspects; (E) Specificity and Kinetics; (F) Where Does the Enzyme: Substrate Reaction Occur? (G) Physiological Aspects; and (H) Enzyme Specificity: Effects of Incubation Time. Clinical implications are also considered in this study as well as implications for therapy and patient management. In a concluding section, the authors point out that means for achieving specific pharmacological and immunologic ablations of surface enzymes are implied by previous discussions of enzyme inhibitors and antibodies prepared against some of the enzymes. Judicious treatment of endothelial cells with enzymes capable of catalyzing limited degradations of glycosaminoglycans, proteoglycans, fibronectin, and some forms of collagen may well yield characteristic molecular scars identifiable via surface replicas. Altogether, prospects seem excellent for adding another detailed chapter to current understanding of cellular and molecular physiology and pathology.

Ryan, J. W. and *Ryan, U. S.*

International Review of Experimental Pathology 26:1-43, 1984.

Other support: U. S. Public Health Service and the John A. Hartford Foundation

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

PROPERTIES OF LATENT AND THIOL-ACTIVATED RAT HEPATIC 3-HYDROXYL-3-METHYLGLUTARYL-COENZYME A REDUCTASE AND REGULATION OF ENZYME ACTIVITY

Previous experiments have suggested to these researchers that thiols activate 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) by binding to it. If a protein-disulfide interchange with the activating thiol does occur, it is readily reversible. In the present report, a simple, quick method for the conversion of soluble Ea to E_a and the kinetics of catalysis by various activation states of HMG-CoA reductase are described. Specifically, the effect of the thiols glutathione (GSH), dithiothreitol (DTT) and dithioerythritol (DTE) on the conversion of an inactive, latent form (E_i) of rat liver HMG-CoA reductase to a catalytically active form (E_a) is examined. Latent hepatic microsomal HMG-CoA reductase is activated to a similar degree of activation by DTT and DTE and to a lower extent by GSH. All three thiols affect both K_m and V_{max} values of the enzyme toward HMG-CoA and NADPH. Studies of the effect of DTT on the affinity binding of HMG-CoA reductase to agarose-hexane-HMG-CoA resin shows that thiols are necessary for the binding of the enzyme to the resin. A combination of these and other results presented here points to the importance of the thiol/disulfide ratio for the modulation of hepatic HMG-CoA reductase activity.

Dotan, I. and *Shechter, I.*

Archives of Biochemistry and Biophysics 226(2):401-410, 1983.

From the Department of Biochemistry, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel.

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REGULATION OF RAT LIVER 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE

This study was undertaken to elucidate the effect of thiols on the modulation of hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity. Specifically, the activation of HMG-CoA reductase by glutathione (GSH) and dithiothreitol (DTT) in rat liver microsomes was studied and compared to the activation by these thiols of enzyme that was solubilized by freeze-thawing. An increase of V_{max} was observed for the two enzyme preparations with increasing concentrations of the two activating thiols. Reactions of GSH-activated microsomal enzyme with increasing concentrations of NADPH show sigmoidal kinetics with a Hill coefficient of 2.01 ±0.07 at 2-4 mM GSH. Increase of the activating GSH concentrations resulted in a gradual change towards Michaelis-Menten kinetics. Activation of the microsomal enzyme by DTT yielded similar results except that a Hill coefficient of 1.1 was observed already at 2.5 mM DTT. Normal Michaelis-Menten kinetics were observed for HMG-CoA at all GSH concentrations. These and other results presented here are compatible with a model in which HMG-CoA reductase activity is GSH-dependent, allosterically modulated under physiological hepatic conditions. In addition, the widely used assay conditions, using high DTT concentrations or employment of highly purified soluble enzyme, precluded the observation of sigmoidal kinetics and the suggested model.

Roitelman, J. and *Shechter, I.*

The Journal of Biological Chemistry 259(2):870-877, 1984.

From the Department of Biochemistry, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel.

MEASUREMENT *IN VIVO* OF IRREVERSIBLE DEGRADATION OF LOW DENSITY LIPOPROTEIN IN THE RABBIT AORTA: PREDOMINANCE OF INTIMAL DEGRADATION

The development of a highly sensitive method for assessing, tissue by tissue, the rates of irreversible protein degradation *in vivo* has allowed the investigators to quantify low density lipoprotein degradation in the normal rabbit aorta and to localize it. The method depends upon the fact that tyramine-cellobiose, like sucrose used in previous studies, can be covalently attached to proteins, enters cells with them, and then remains trapped within the cell after the remainder of the protein molecule has been degraded. Rabbit LDL (d = 1.02 to d = 1.06 g/ml) was labeled with ^{125}I-tyramine-cellobiose and injected into rabbits. Aortic ^{125}I content 24 hours later served as a cumulative measure of degraded LDL (after appropriate corrections for any intact, nondegraded LDL present). Calculated aortic degradation of LDL averaged 9.4×10^{-3} percent of the plasma pool per g aortic wet weight per day (n = 6). Intimal cells, obtained by gentle swabbing, accounted for fully 40% of total aortic degradation even though the intima represented less than 5% of the aortic mass. Autoradiography confirmed the high concentration of label in the intima. Degradation of unmodified and reductively methylated LDL were compared. The fractional rate of degradation of methylated LDL by the intima was 50-60% of that for native LDL, indicating that 40-50% of LDL degradation in the intima, predominantly endothelial cells, is mediated by LDL receptors.

Carew, T. E., Pittman, R. C

Arteriosclerosis 4:214-224, 1

Other support: National He

From the Division of Meta California, San Diego, La J

α-ADRENERGIC-MEDIA SECONDARY TO CAROT CONSCIOUS DOGS

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Murray, P. A., Lavallee, M

Circulation Research 54(1)

Other support: U. S. Public American Heart Associatio

From the Departments of Women's Hospital, Bost Center, Southboro, MA.

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Carew, T. E., Pittman, R. C., Marchand, E. R., and *Steinberg, D.*

Arteriosclerosis 4:214-224, 1984.

Other support: National Heart, Lung and Blood Institute.

From the Division of Metabolic Disease, Department of Medicine, University of California, San Diego, La Jolla.

α-ADRENERGIC-MEDIATED REDUCTION IN CORONARY BLOOD FLOW SECONDARY TO CAROTID CHEMORECEPTOR REFLEX ACTIVATION IN CONSCIOUS DOGS

The investigators examined the late coronary vascular response to carotid chemoreceptor reflex activation in normal, conscious dogs instrumented for the measurement of right main and left circumflex coronary artery blood flows, arterial and right ventricular pressures, and arterial and coronary sinus blood gases and O_2 contents. With heart rate held constant by electrical stimulation, and with respiration controlled or allowed to vary spontaneously, carotid chemoreceptor reflex activation (induced by intracarotid nicotine) elicited a striking biphasic coronary vascular response characterized by an early dilation (previously described) and a late constriction. For example, with respiration controlled and with the autonomic nervous system intact, carotid chemoreceptor reflex activation resulted in a late increase in arterial pressure (19 ± 4%; $P<0.002$), absolute reductions in right main (24 ± 4%; $P<0.002$), and left circumflex (12 ± 2%; $P<0.004$) coronary blood flows, and increases in right (62 ± 13%; $P<0.002$) and left (26 ± 3%; $P<0.0001$) coronary resistances. This carotid chemoreceptor reflex activation-induced late coronary constriction was also associated with a concomitant increase in myocardial oxygen extraction, i.e., arterial oxygen content remained constant while coronary sinus oxygen content decreased (19 ± 6%; $P<0.04$). Neither propranolol nor atropine had any significant effect on the magnitude of the right coronary constriction. However, both the absolute reduction in right coronary blood flow and increase in right coronary resistance were abolished by phentolamine. Furthermore, either total cardiac denervation or adrenalectomy significantly attenuated ($P<0.01$) carotid chemoreceptor reflex activation-induced reductions in right coronary blood flow and increase in right coronary resistance. We conclude that, with autonomic nervous system activity intact, carotid chemoreceptor reflex activation can elicit an absolute reflexly mediated reduction in coronary blood flow in the normal, conscious dog despite an increase in arterial pressure. The mechanism of the vasoconstriction involves α-adrenergic receptor stimulation mediated by both cardiac sympathetic nerves and circulating catecholamines.

Murray, P. A., Lavallee, M. and *Vatner, S. F.*

Circulation Research 54(1):96-106, 1984.

Other support: U. S. Public Health Service, Massachusetts Heart Association and the American Heart Association.

From the Departments of Medicine, Harvard Medical School, and Brigham and Women's Hospital, Boston, and the New England Regional Primate Research Center, Southboro, MA.

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Source: https://www.industrydocuments.ucsf.edu/docs/ypxk0000

REFLEX CARDIOVASCULAR RESPONSES TO CHEMORECEPTOR STIMULATION IN CONSCIOUS DOGS WITH CARDIAC HYPERTROPHY

This paper contains the first data to describe the effects of severe RV hypertrophy on reflex cardiovascular responses to carotid chemoreceptor reflex activation (CCRA). α-adrenergic-mediated reflex constriction of the iliac arterial vascular bed in response to CCRA appears normal, whereas the cardiac cycle length response to this reflex stimulus is enhanced in dogs with RV hypertrophy. In striking contrast, an apparent selective abnormality in reflex α-adrenergic vasoconstriction of the coronary vasculature supplying the hypertrophied right ventricle has been documented.

Murray, P. A., and *Vatner, S. F.*

American Journal of Physiology 245(Heart Circ. Physiol.) 14:H871-H879, 1983.

Other support: National Heart, Lung and Blood Institute, Massachusetts Heart Association and the American Heart Association.

From the Department of Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, and the New England Regional Primate Research Center, Southboro, MA.

ALPHA-ADRENERGIC REGULATION OF THE CORONARY CIRCULATION IN THE CONSCIOUS DOG

To examine the role of neural and humoral factors in the control of coronary vasoactivity in conscious animals in which the complicating effects of general anesthesia and recent surgery were absent, dogs were instrumented with miniature pressure gauges in the aorta and left ventricle to measure aortic and left ventricular pressures and with flow probes in the left circumflex coronary artery to measure coronary blood flow. Several weeks after recovery from operation, experiments were conducted in the conscious dogs. Stimulation of the carotid chemoreceptor reflex elicited a biphasic response; initially striking reflex coronary vasodilation was observed: Coronary blood flow tripled even after changes in metabolic factors were minimized by pretreatment wtih propranolol. The chemoreceptor reflex coronary vasodilation was mediated both by cholinergic activation and withdrawal of alpha-adrenergic tone. The second phase of the response involved an increase in coronary vascular resistance, associated with elevated arterial pressure and an absolute reduction in coronary blood flow and coronary sinus oxygen content. The later period of coronary vasoconstriction was abolished by alpha-adrenergic blockade. Intravenous infusion of the alpha-adrenergic agonist methoxamine increased mean arterial pressure by 65 ± 5% and total coronary vascular resistance by 92 ± 14%. To determine whether the large coronary arteries were also under autonomic control, the dogs were instrumented with a pair of ultrasonic crystals on opposing surfaces of the left circumflex coronary artery to measure its diameter directly and continuously. Methoxamine reduced coronary cross-sectional area substantially, thereby constricting large coronary arteries and resistance coronary vessels. Thus, while the coronary arteries are regulated by the changes in myocardial metabolic demands, they are also responsive to changes in neural stimuli. The most striking changes were observed with augmentation and withdrawal of alpha-adrenergic constrictor tone, which appears to be important in the control of large as well as small coronary vessels.

Vatner, S. F.

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Vatner, S. F.

The American Journal of Cardiology 52: 15A-21A, 1983.

U.S. Public Health Service.

From the Departments of Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, and the New England Regional Primate Research Center, Southboro, MA.

CONTRIBUTIONS OF HUMAN PLATELETS TO THE PROTEOLYTIC ACTIVATION OF BLOOD COAGULATION FACTORS XII AND XI

Previous studies have suggested that human platelets can promote the activation of factor XI by two different mechanisms, one requiring factor XII and ADP-treated platelets and the other requiring collagen-treated platelets in the apparent absence of factor XII. To investigate these hypotheses, isolated platelets were tested for their capacity to promote the activation and cleavage of purified factors XII and XI in various mixtures of purified factor XII, kallikrein, high molecular weight (HMW) kininogen, and factor XI. The activation of factor XII or factor XI was tested in clotting assays using plasmas deficient in factor XII, XI, IX, or VII and the limited proteolytic cleavage of ^{125}I-labeled factor XII or XI was assessed using sodium dodecyl sulfate polyacrylamide gel electrophoresis in the presence of reducing agents. That ADP- or collagen-treated platelets can promote the proteolytic activation of factor XII in mixtures containing kallikrein and HMW kininogen was shown by: (1) the proteolytic cleavage of factor XII; (2) the development of factor XII, coagulant activity; and (3) the proteolytic cleavage of ^{125}I-factor XI. Platelets treated with collagen or thrombin were shown by both coagulant assays and by cleavage studies to participate with HMW kininogen and kallikrein in the proteolytic activation of Factor XI by mechanisms that were partially dependent upon and partially independent of factor XII. That these zymogen activations are platelet-related was demonstrated by: (1) the enhanced coagulant activity and proteolysis observed after platelet stimulation with ADP, collagen or thrombin; (2) the inhibition of proteolytic activation seen when platelet responses were inhibited by indomethacin; (3) the absence of proteolytic activation in the absence of platelets or other surfaces; and (4) the localization of cleavage products of factor XI in platelet pellets. Experiments with celite or with unstimulated platelets present showed a linear relationship between factor-XII coagulant activity and proteolysis of factor XII. However, with activated platelets present the coagulant activity was two to fourfold greater than that observed for an equivalent amount of factor XII cleaved with celite present. These studies demonstrate that platelets can promote the proteolytic activation of factor XII by kallikrein and of factor XI by both factor-XII dependent and factor-XII-independent mechanisms.

Walsh, P. N. and Griffin, J. H.

Blood 57(1):106-118, 1981.

Other support: National Institutes of Health.

From the Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, CA, and the Specialized Center on Thrombosis Research, Department of Medicine, Temple University School of Medicine, Philadelphia.

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Source: https://www.industrydocuments.ucsf.edu/docs/ypxk0000

NEUTROPHIL-ASSISTED DNA SYNTHESIS BY HUMAN LYMPHOCYTES IN RESPONSE TO MEVALONIC ACID; ENHANCEMENT BY CYTOCHALASIN B

Mevalonic acid, a key intermediate in the biosynthesis of cholesterol, is capable of initiating DNA synthesis, morphologic transformation and cell division in cultures of human peripheral blood lymphocytes. Studies from this laboratory show that pure populations of lymphocytes respond poorly to mevalonic acid, but their response can be enhanced by peripheral blood neutrophils. Addition of cytochalasin B, but not cytochalasin A, to mixed neutrophil-lymphocyte cultures enhances the response of lymphocytes to both Con A and mevalonate, but the increment in mevalonate-induced DNA synthesis far exceeds the enhancement which cytochalasin B produces in the Con A response. As a consequence, the DNA synthetic response in mevalonate containing cells averages 39% of the response to an optimal dose of Con A. These, and several related experiments, suggest that the enhancing effect of cytochalasin B in the response of mixed neutrophil-lymphocyte cultures to mevalonic acid results from its ability to potentiate a time-dependent conditioning effect on lymphocytes which neutrophils exert. To sum up, the ability of mevalonic acid to initiate DNA synthesis and cell cycling in normal and abnormal cells, and the possibility that a nonsterol product of mevalonate metabolism may be the mediator of this effect, adds to the growing knowledge about the relationship between mevalonate metabolism, DNA synthesis and cell replication.

Larson, R. A., Gordon, L. I., and *Yachnin, S.*

Cellular Immunology 81:357-372, 1983.

Other support: U. S. Public Health Service, Nalco Cancer Research Fund, Leukemia Society of America, and the American Cancer Society.

From the Department of Medicine and the Committee on Immunology, the University of Chicago School of Medicine, and the Department of Medicine, Northwestern Medical Center, Chicago.

DIVERGENCE IN CHOLESTEROL BIOSYNTHETIC RATES AND 3-HYDROXY-3-METHYLGLUTARYL-COA REDUCTASE ACTIVITY AS A CONSEQUENCE OF GRANULOCYTE VERSUS MONOCYTE-MACROPHAGE DIFFERENTIATION IN HL-60 CELLS

The various classes of leukocytes in human peripheral blood differ significantly from each other with respect to cholesterol biosynthesis. In this report, the HL-60 cell line was used to examine and compare the changes in the cholesterol biosynthetic pathway associated with granulocytic or monocyte-macrophage differentiation. Results show that dimethyl sulfoxide-induced granulocyte differentiation is associated with a decrease in cellular 3-hydroxy-3-methylglutaryl (HMG-CoA) reductase activity and with a decrease in the incorporation of [^{14}C] acetate and mevalonate into products of the cholesterol biosynthetic pathway. Phorbol myristate acetate (PMA)-induced monocyte-macrophage differentiation in these cells is associated with a rapid and profound fall in cell proliferation, but nonetheless is accompanied by a dose-dependent increase in cellular HMG-CoA reductase activity and [^{14}C] acetate incorporation into the cholesterol biosynthetic pathway. In addition, PMA induces an increase in [^{14}C] mevalonate incorporation into cholesterol and its precursors, suggesting that post-

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cate that this divergence in ster
normally also derive from a co
differentiation process.

Yachnin, S., Toub, D. S. and

Proceedings of the National
81:894-897, 1984.

Other support: U. S. Public

From the Department of Med
of Chicago, Chicago.

INCREASED 3-HYDROXY
REDUCTASE ACTIVITY
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In this study of choleste
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Yachnin, S. and Mannickar

Blood 63(3):690-693, 1984

Other support: U. S. Publ

From the Department of M
of Chicago, Chicago.

MEVALONIC ACID IND
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Recent studies have
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Source: https://www.industrydocuments.ucsf.edu/docs/ypxk0000

HMG-CoA reductase events in cholesterol biosynthesis are also enhanced. Mature peripheral blood human monocytes possess an active cholesterol biosynthetic pathway, whereas mature human granulocytes are almost entirely lacking in the ability to synthesize post-squalene products. These investigators' results with HL-60 cells indicate that this divergence in sterol-synthesizing ability between two cell lineages, which normally also derive from a common stem cell, can be observed as an early event in the differentiation process.

Yachnin, S., Toub, D. S., and Mannickarottu, V.

Proceedings of the National Academy of Sciences of the United States of America 81:894-897, 1984.

Other support: U. S. Public Health Service.

From the Department of Medicine and the Committee on Immunology, the University of Chicago, Chicago.

INCREASED 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE ACTIVITY AND CHOLESTEROL BIOSYNTHESIS IN FRESHLY ISOLATED HAIRY CELL LEUKEMIA CELLS

In this study of cholesterol synthesis, freshly isolated hairy cells from the peripheral blood of patients with hairy cell leukemia (HCL) synthesized 3-5-fold greater amounts of cholesterol, lanosterol and squalene from [1-^{14}C]-acetate that did normal human peripheral blood mononuclear cells under basal conditions of culture (i.e., in the presence of low-density lipoprotein). HCL cells also exhibited an eightfold increase in the activity of the enzyme 3-hydroxyl-3-methylglutaryl coenzyme A reductase. These changes cannot be ascribed to increased rates of cellular proliferation in the HCL cells, nor are they a consequence of an increased rate of loss of newly synthesized cholesterol into the culture medium. The increased rate of cholesterol biosynthesis in HCL cells may result in an increase in their total cellular cholesterol content, as well as in an increase in their plasma membrane cholesterol:phospholipid molar ratio. These changes, in turn, are probably responsible for some of the clinical manifestations of this disease.

Yachnin, S. and Mannickarottu, V.

Blood 63(3):690-693, 1984.

Other support: U. S. Public Health Service.

From the Department of Medicine and the Committee on Immunology, the University of Chicago, Chicago.

MEVALONIC ACID INDUCES DNA SYNTHESIS IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

Recent studies have demonstrated the essential role of mevalonic acid in the regulation of mammalian cell DNA synthesis and cell division and have suggested that a mevalonate-derived nonsterol growth factor may be involved. In the study presented here it was seen that mevalonic acid can stimulate leukemic cells from some patients with B cell chronic lymphocytic leukemia (CLL) to enter the cell cycle *in vitro* and to

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Source: https://www.industrydocuments.ucsf.edu/docs/ypxk0000

synthesize DNA. Unlike normal human peripheral blood lymphocytes, which require the presence of granulocytes for a maximal DNA synthetic response to mevalonic acid, CLL cells do not require a helper cell. Only the physiologically active R(−) enantiomer of mevalonic acid is active in initiating DNA synthesis, and no stimulatory effect is noted after addition of the precursors of mevalonic acid or of its known products. The mitogenic responses to mevalonic acid measured in CLL cells from 35 patients varied widely (range of stimulation indices, 1.2-18.6), but the DNA synthetic response of CLL cells from nine patients to mevalonic acid exceeded those seen with such common lymphocyte mitogens as concanavalin A, phytohemagglutinin, pokeweed mitogen, or a phorbol ester. The mevalonate-responsive CLL cell was enriched in the E(−), M(+) rosette subpopulation. When CLL cells were incubated in lipoprotein-depleted serum-containing medium, the presence of low density lipoprotein cholesterol increased their sensitivity to mevalonic acid transformation, suggesting that a nonsterol product of mevalonic acid metabolism may be involved. Mevalonic acid, which is essential for the growth of cells programmed to divide or stimulated to divide by mitogens, can by itself initiate cell replication in relatively inert G_0 phase normal and neoplastic lymphocyte populations.

Larson, R. A. and *Yachnin, S.*

Blood 64(1):257-262, 1984.

Other support: U. S. Public Health Service and the Nalco Cancer Research Fund.

From the Department of Medicine, University of Chicago, Chicago.

IV. *Neuropharmacology and Physiology*

TRANSIENT EXPRESSION OF SELECTED CATECHOLAMINERGIC TRAITS IN CRANIAL SENSORY AND DORSAL ROOT GANGLIA OF THE EMBRYONIC RAT

One outstanding problem in developmental neurobiology concerns the regulation of neuronal phenotypic expression *in vivo*. The present study addresses this problem by describing cellular populations, located in embryonic cranial sensory and dorsal root ganglia, which transiently express catecholamine-specific enzymes *in vivo*. Isolated cells expressing tyrosine hydroxylase (T-OH) immunoreactivity were initially detected in trigeminal (V) ganglion anlages as early as gestation day 10.5 (E10.5; 18-22 somites). Neurofilament (NF) protein was also evident in V at these early stages. By E11.5 (27-30 somites) clusters of T-OH-positive cells were visible in V. Many of these cells were bipolar sending processes into the primitive brainstem. In addition, cells expressing T-OH were apparent in primordia of sensory ganglia serving the glossopharyngeal (IX) and vagal (X) cranial nerves, as well as in rostral dorsal root ganglia. Unlike transiently catecholaminergic cells of the developing intestine, these cells lacked demonstrable catecholamine histofluorescence and a high-affinity uptake process. By this stage (E11.5) all cranial sensory ganglia were rich in NF protein, but immunoreactivity was confined to cellular processes rather than perikarya. By E13.5,

T-OH was absent from all se
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Jonakait, G. M., Markey, K.

Developmental Biology 101(1

Other support: National Inst

From the Division of Develop
and the Department of Psych

DIFFERENTIAL EFFECTS
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This study evaluated
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Andersson, K., *Fuxe, K.*

Acta Physiologica Scandi

Other support: Svenska

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and the Department of H

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Source: https://www.industrydocuments.ucsf.edu/docs/ypxk0000

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Cancer Research Fund.

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han perikarya. By E13.5,

T-OH was absent from all sensory ganglion neurons. These and other observations suggest that transient expression of neurotransmitters during development is widespread, occurring in cells of diverse origins and fates.

Jonakait, G. M., Markey, K. A., Goldstein, M., and *Black, I. B.*

Developmental Biology 101(1):51-60, 1984.

Other support: National Institutes of Health and the Dysautonomia Foundation.

From the Division of Developmental Neurology, Cornell Medical College, New York, and the Department of Psychiatry, New York University Medical Center, New York.

DIFFERENTIAL EFFECTS OF MECAMYLAMINE ON THE NICOTINE INDUCED CHANGES IN AMINE LEVELS AND TURNOVER IN HYPOTHALAMIC DOPAMINE AND NORADRENALINE NERVE TERMINAL SYSTEMS AND IN THE SECRETION OF ADENOHYPOPHYSEAL HORMONES IN THE CASTRATED FEMALE RAT. EVIDENCE FOR INVOLVEMENT OF CHOLINERGIC NICOTINE-LIKE RECEPTORS

This study evaluated the effects of mecamylamine on the nicotine induced changes in hypothalamic catecholamine (CA) levels and turnover in female rats ovariectomized for one month. In the same group of animals, the serum prolactin, LH, FSH, TSH, GH, and corticosterone levels were measured using radioimmunoassay procedures. Results showed that the nicotine treatment induced a significant depletion of amine stores and an increase of amine turnover in dopamine and noradrenaline (NA) nerve terminals of the median eminance and of the peri- and paraventricular and dorsomedial NA systems of the hypothalamus using the tyrosine hydroxylase inhibition model. Mecamylamine partly counteracted the nicotine induced reduction of amine stores in peri- (anterior part) and paraventricular NA nerve terminal systems as well as the nicotine induced increase of NA turnover in these systems, but not the action of nicotine on the CA systems of the median eminence. Nicotine significantly and markedly reduced prolactin, LH, TSH, and GH secretion and increased corticosterone secretion, but did not influence FSH secretion. These effects were partly counteracted by mecamylamine in the case of prolactin, LH and TSH secretion but not in the case of GH and corticosterone secretion. Overall, the discovered differential blockade of mecamylamine on nicotine induced change in CA levels and turnover in the paraventricular area and the anterior periventricular hypothalamic region as compared with the median eminence region opens up the possibility of the existence of different types of cholinergic nicotine-like receptors with different susceptibility to the blocking activity of mecamylamine and possibly other blockers of cholinergic nicotine-like receptors.

Andersson, K., *Fuxe, K.*, Eneroth, P., and Agnati, L. F.

Acta Physiologica Scandinavica 120:489-494, 1984.

Other support: Svenska Tobaks AB, Stockholm.

From the Department of Histology, Karoklinska Institutet, Stockholm, Hormone Laboratory, Department of Obstetrics and Gynaecology, Karolinska Hospital, Stockholm, and the Department of Human Physiology, University of Modena, Modena, Italy.

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Source: https://www.industrydocuments.ucsf.edu/docs/ypxk0000

EFFECT OF ETHANOL ON CHOLECYSTOKININ-INDUCED ENZYME SECRETION FROM ISOLATED RAT PANCREATIC ACINI

Cholecystokinin octapeptide (CCK_8)-stimulated amylase release in isolated rat pancreatic acini was inhibited over 30% by 600 mM ethanol. The configuration of the dose-response curve for CCK_8, however, in the presence of the ethanol was similar to that of the control. Amylase release elicited by maximal concentrations of CCK_8 (300 pM) was inhibited by increasing concentrations of ethanol (0.3 to 1.3 M), and this inhibition was concentration dependent. In addition, the binding of [^{125}I]CCK_{33} to specific membrane receptors on acini was inhibited by ethanol in a dose-dependent manner. A positive correlation between the inhibitory effects of ethanol on CCK binding and CCK-induced amylase release was observed. Furthermore, these inhibitory effects of ethanol were reversible. Basal amylase release, however, was increased 20-50% by ethanol between the concentrations of 0.3 and 1.3 M; higher concentrations caused a leakage of amylase from the acini both in the absence and presence of 300 pM CCK_8. This is confirmed by ^{51}Cr release from prelabeled acini which revealed no significant damage to acinar cell membrane between 0.3 and 1.6 ethanol, but significant damage to acini at higher concentrations. These data suggest that the 600 mM ethanol-induced inhibition of CCK action in acini is due to reversible perturbation of the acinar cell membrane.

Lewin, M. B., Sankarae, H., DeVeney, C. W., Wong, A., Wendland, M. F., and *Geokas, M. C.*

Biochemical Pharmacology 33(20):3225-3229, 1984.

Other support: Medical Research Service of the Veterans Administration.

From the Department of Medicine, University of California, Davis; Enzymology Research Laboratory, VA Medical Center, Martinez, CA; and Surgical Service, VA Medical Center and Department of Surgery, University of California, San Francisco.

REDUCTION OF DOPAMINE UPTAKE AND COCAINE BINDING IN MOUSE STRIATUM BY N-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE

N-Methyl-4-phenyl-1,2,3,6-tetrahydropyrine (NMPTP) has been shown earlier to have toxic effects on the nigrostriatal dopamine system in the rhesus monkey, with biochemical, histological and behavioral symptoms similar to Parkinsonism. In the study presented here, administration of NMPTP (daily injections of 8 mg/kg for five days via tail vein) reduced [^{3}H]dopamine uptake in striatal synaptosomes by 63% and reduced [^{3}H]cocaine binding to striatal membranes by 61%. [^{3}H]cocaine binding was not affected in olfactory tubercle, suggesting a selective effect of NMPTP on the nigrostriatal but not on the mesolimbic dopaminergic system. The destruction of dopamine terminals in the striatum did not alter (up-regulate) [^{3}H]spiroperidol binding. The present results suggest that NMPTP has degenerative effects on dopaminergic pathways in the nigrostriatal pathway in mice and that it may be useful in developing a rodent model of Parkinson's disease.

Sershen, H., Reith, M. E. A., Hashim, A., and *Lajtha, A.*

European Journal of Pharmacology 102:175-178, 1984.

Other support: National Science Foundation.

From the Center for Neurochemistry, Ward's Island, New York.

FACTORS AFFECTING THE
PLACENTAL VILLUS: ACET
METHYLATION, CA^{++} AND

Placental insufficiency for
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Sastry, B. V. Rama, Barnwell,

Trophoblast Research 1:81-100,

Other support: U.S. Public He

From the Department of Pharm
Nashville, TN.

DEPRESSION OF AMINO AC
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As summarized in this pap
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Source: https://www.industrydocuments.ucsf.edu/docs/ypxk0000

FACTORS AFFECTING THE UPTAKE OF α-AMINO ACIDS BY HUMAN PLACENTAL VILLUS: ACETYLCHOLINE, PHOSPHOLIPID METHYLATION, Ca^{++} AND CYTOSKELETAL ORGANIZATION

Placental insufficiency for the transport of amino acids may contribute to intrauterine fetal growth retardation. Therefore, effects of several factors on the uptake of amino acids by isolated placental villus using mainly the nonmetabolizable α-aminoisobutyric acid (AIB) were studied. In a limited number of experiments, aspartic acid, isoleucine and lysine were used. The following results were obtained: (1) 2-Benzoylethyltrimethylammonium (BETA), an inhibitor of choline acetyltransferase (ChA), inhibited AIB uptake, lowered ChA and acetylcholine (ACh) levels and decreased ACh release. (2) BETA inhibited incorporation of metabolizable amino acids into proteins, possibly by lowering ACh levels. (3) α-Naphthoylethyl-trimethylammonium (α-NETA) inhibited ChA and AIB uptake by placental villus. There was a positive relationship between the inhibition of ChA and the depression of AIB uptake by the villous tissue. The inhibition of placental ChA by α-NETA was not reversed by washing the tissue or by dialyzing the homogenates. (4) Placental villus contains two phospholipid N-methyltransferases (PMT I and II). In the presence of S-adenosyl-L-methionine, PMT I converted phosphatidylethanolamine into phosphatidyl-N-methylethanolamine (PME), and PMT II converted PME into phosphatidylcholine. Inhibiting PMTs in plasma membrane by increasing the intracellular levels of S-adenosyl-L-homocysteine (SAH) in the placental villus inhibited AIB uptake. (5) Ca^{++} is required for both release of ACh and AIB uptake. (6) Inhibition of the microtubular assembly by colchicine depressed AIB uptake. (7) Whole placental trophoblast cells are necessary for efficient active uptake of AIB. These observations indicate that (1) ACh synthesis, its release and its activation of the cholinergic receptor; (2) membrane phospholipid methylation, Ca^{++} transport and amino acid transport sytems; and (3) an organized cytoskeletal system are linked to one another for effective transport of AIB across human placental trophoblast.

Sastry, B. V. Rama, Barnwell, S. L. and Moore, R. D.

Trophoblast Research 1:81-100, 1983.

Other support: U.S. Public Health Service.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

DEPRESSION OF AMINO ACID UPTAKE IN HUMAN PLACENTAL VILLUS BY COCAINE, MORPHINE AND NICOTINE

As summarized in this paper, amino acid uptake was measured *in vitro* in intact human placental villus. Active uptake of α-aminoisobutyric acid (AIB), aspartic acid, isoleucine and lysine was demonstrated. The inhibition of the uptake of these amino acids was studied using cocaine, morphine and nicotine. The uptake of AIB was most sensitive to inhibition by these agents at millimolar concentrations, demonstrating that the different amino acid transport systems may be affected differently by these drugs. The metabolism of aspartic acid, isoleucine and lysine in the placenta may be slightly decreased by these drugs. The inhibition of AIB uptake by these drugs was neither simple competitive nor noncompetitive. The uptake of AIB depends on Ca^{++} in the p

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Source: https://www.industrydocuments.ucsf.edu/docs/ypxk0000

medium. These findings suggest that the placental cholinergic function, by decreasing ACh release, may affect amino acid uptake.

Barnwell, S. L. and *Sastry, B. V. Rama.*

Trophoblast Research 1:101-120, 1983.

Other support: U. S. Public Health Service.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

V. *Pharmacology and Biochemistry*

CLATHRIN LIGHT CHAINS AND SECRETORY VESICLE BINDING PROTEINS ARE DISTINCT

Recently, several groups of investigators have initiated studies on cytosolic proteins that bind to isolated secretory vesicle membranes in the presence of Ca^{++} in order to identify proteins that may regulate exocytosis. Two major chromaffin granule binding proteins, of molecular weights 32,000 (32K) and 34,000 (34K), were reported to have the same mobility on one-dimensional SDS gels as clathrin-associated light chains from the adrenal medulla, and the 34K granule binding protein the same one-dimensional peptide map as the 34K clathrin light chain. These observations support the hypothesis that Ca^{++}-dependent recruitment of soluble light chains to the vesicle membrane may nucleate the assembly of a clathrin coat and initiate endocytosis. However, it is reported here that two-dimensional peptide maps of the clathrin light chains and of all chromaffin granule membrane binding proteins in the 30K range are distinct and, therefore, fail to support this hypothesis. It has also been suggested that some or all of the vesicle binding proteins require calmodulin for their interaction with the membrane. However, it was found that an antagonism of calmodulin by trifluoperazine does not prevent the association of the other cytosolic proteins with the chromaffin granule membrane.

Creutz, C. E. and Harrison, J. R.

Nature 308(5955):208-210, 1984.

Other support: National Science Foundation, the Virginia Affiliate of the American Heart Association, and the University of Virginia Diabetes Research and Training Center.

From the Department of Pharmacology, University of Virginia School of Medicine, Charlottesville.

ON THE CYTOTOXICITY OF
SITE-SPECIFIC FENTON MEC

In the work presented here,
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Samuni, A., Aronovitch, J., Go

European Journal of Biochemis

Other support: U. S. Departme
Neuherberg, West Germany.

From the Departments of Mol
Chemistry, Hebrew University

MECHANISMS OF THE DISM
THE COPPER(II) PHENANTH
OF THE COPPER(I) PHENAN
AQUEOUS SOLUTION

In view of the role of $(op)_2C$
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Source: https://www.industrydocuments.ucsf.edu/docs/ypxk0000

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ON THE CYTOTOXICITY OF VITAMIN C AND METAL IONS: A SITE-SPECIFIC FENTON MECHANISM

In the work presented here, the toxicity of ascorbate towards phage lambda and the phages T2-T7 has been investigated. At room temperature the T-odd and lambda bacteriophages are highly susceptible to ascorbate-induced damage, whereas the T-even phages are practically resistant. The toxicity of ascorbate is dependent on the presence of copper (or iron) and oxygen, although oxygen is not required in the presence of H_2O_2. Hydrogen peroxide is essential for the ascorbate-induced phage inactivation and the damage is prevented by catalase. At the concentrations used, most of the copper ions are bound to the phage particles. Chelating agents such as EDTA or histidine fully protect the phages, whereas salicylate only reduces the rate of phage inactivation. OH scavengers such as sucrose, formate, mannitol, *tert*-butyl alcohol or poly(ethylene glycol) have no protective effect. In addition, experiments with DNA-labeled phages indicate that both phage adsorption and DNA injection are impaired as a result of the exposure to ascorbate and copper. The failure to express the viral genetic information as a result of single- and double-strand breaks in the DNA probably also contribute to the loss of the plaque-forming ability of the phages. These results are interpreted in terms of a "site-specific" Fenton mechanism according to which the binding of the transition metal ions to the biological target is a prerequisite for the production of damage. The bound metal ion is reduced either by O_2^-, ascorbate or other reductants and is subsequently reoxidized by H_2O_2 yielding OH^- radicals which react with vital macromolecules with a high probability of causing "multi-hit" damage. This "site-specific" formation of OH^- radicals, which takes place near the target molecules, accounts both for the high damaging efficiency and for the failure of OH^- scavengers to protect against it.

Samuni, A., Aronovitch, J., Godinger, D., Chevion, M., and *Czapski, G.*

European Journal of Biochemistry 137:119-124, 1983.

Other support: U. S. Department of Energy and Gesellschaft für Strahlenforschung Neuherberg, West Germany.

From the Departments of Molecular Biology, Cellular Biochemistry and Physical Chemistry, Hebrew University of Jerusalem, Israel.

MECHANISMS OF THE DISMUTATION OF SUPEROXIDE CATALYZED BY THE COPPER(II) PHENANTHROLINE COMPLEX AND OF THE OXIDATION OF THE COPPER(I) PHENANTHROLINE COMPLEX BY OXYGEN IN AQUEOUS SOLUTION

In view of the role of $(op)_2Cu^{2+}$ in the degradation of DNA, and the possible role of O_2^- in this process, the authors decided to study the catalytic effect of $(op)_2Cu^{2+}$ upon the disproportion of O_2^- and the kinetics and the reaction mechanism of the oxidation of $(op)_2Cu^{2+}$ by molecular oxygen in the presence and in the absence of H_2O_2. By using the technique of pulse radiolysis to generate O_2^-, it is demonstrated here that copper 1,10-phenanthroline $(op)_2Cu^{2+}$ is capable of catalytically dismutating O_2^- with a "turnover" rate constant $k_{cat} = (5.1 \pm 0.9) \times 10^8\ M^{-1}\ s^{-1}$. The rate constants of the reduction of $(op)_2Cu^{2+}$ and of the oxidation of $(op)_2Cu^{2+}$ by O_2^- have been determined to be (1.93 ±

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0.07) × 10^8 and (2.95 ± 0.3) × 10^8 M^{-1} s^{-1}, respectively. The kinetic results of the oxidation of (op)$_2Cu^{2+}$ by molecular oxygen in aqueous solution are interpreted by a mechanism that proceeds via a superoxide intermediate, and the rate constant k-10 = (5.0 ± 0.3) × 10^4 M^{-1} s^{-1} has been determined. The rate constant of the oxidation of (op)$_2Cu^{2+}$ by H_2O_2 was measured to be k_{32} = (937 ± 20) M^{-1} s^{-1}.

Goldstein, S. and *Czapski, G.*

Journal of the American Chemical Society 105(25):7276-7280, 1983.

Other support: U. S. Department of Energy.

From the Department of Physical Chemistry, the Hebrew University of Jerusalem, Jerusalem, Israel.

ROLES OF COPPER AND O_2^- IN THE RADIATION-INDUCED INACTIVATION OF T7 BACTERIOPHAGE

As reported in this paper, the effect of copper on the radiation damage induced in T7 bacteriophage has been investigated. The phages were α-irradiated and the effects of copper(II) ions in the presence of various additives and radical scavengers were examined in an attempt to better understand the effect of transition metal ions on the role of free radicals, particularly superoxide, in biological damage. This work extends a study previously done on isolated enzyme to a whole biological entity. Copper(II) ions, even at very low concentrations, enhanced the lethal effect of radiation. This sensitization was observed in both the presence and absence of oxygen. The effect of copper could be reverted by chelating agents such as EDTA or 1,10-phenanthroline. Hydrogen peroxide enhanced the sensitizing effect of copper, though little if any protection was provided by catalase or SOD. High molecular weight scavengers of free radicals in the presence of both copper(II) and hydrogen peroxide had no protective effect. (This is in contrast to metal-free systems where, although such scavengers are incapable of penetrating the phages, they protect them against inactivation.) Low molecular weight scavengers of free radicals reduced but did not eliminate the sensitizing effect of copper. The sensitizing effect was also observed with other T-odd phages, but not with the T-even series. The results are interpreted in terms of a site-specific Fenton mechanism according to which the binding of the metal ion to the phages is a prerequisite for the occurrence of the biological damage. The results also indicate that most of the copper effect is *endogenous*. This is in accord with the failure of copper to sensitize the T-even phages, which differ by the rigidity and permeability of their outer coat structures.

Samuni, A., Chevion, M. and *Czapski, G.*

Radiation Research 99:562-572, 1984.

Other support: United States-Israel Binational Science Foundation and the Gesellschaft fur Strahlenforschung Neuherberg, West Germany.

From the Department of Molecular Biology, Department of Cellular Biochemistry, and Department of Physical Chemistry, the Hebrew University of Jerusalem, Israel.

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